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MANGOSTEEN PEEL EXTRACT (MPE) AND DFDBBX APPLIED TO CAVIA COBAYA TOOTH EXTRACTION SOCKETS FOR GUIDED BONE REGENERATION

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ABSTRACT

Objectives: The objective of this study was to examine the clinical and microbiological effects of combining mangosteen peel extract (MPE) and demineralized freeze-dried bovine bone xenograft (DFDBBX) on the expression of fibroblast growth factor-2 (FGF-2), the amount of osteoblast, and the amount of osteoclast in the alveolar bone following Cavia cobaya tooth extraction. Tooth extraction followed by alveolar ridge resorption is an unavoidable physiological process. The combination of MPE and DFDBBX is expected to primarily provide osteoconductive properties as well as strengthen the graft in order to stimulate new bone formation. **Materials and Methods:** Fifty-six Cavia cobaya were divided into two groups for 7-day (group A) and 30-day (group B) examinations, respectively. Each group consisted of twenty-eight animals that received four treatments. In each treatment, seven Cavia cobaya were used. The bottom right incisor was extracted. In groups AI and BI, tooth sockets were filled with 25 grams of polyethylene glycol (PEG). Tooth sockets in groups AII and BII were filled with 0.5 grams of DFDBBX and 24.5 grams of PEG. Tooth sockets in groups AIII and BIII were filled with 0.5 grams of MPE and 24.5 grams of PEG. In groups AIV and BIV, tooth sockets were filled with a mixture of 0.5 grams of DFDBBX, 0.5 grams of MPE, and 24 grams of PEG. Cavia cobaya's mandible was decalcified with ethylene diamine tetraacetic acid (EDTA) at 7 and 30 days. During this time, histopathology (HPA) and immunohistochemistry (IHC) tests were also performed. **Statistical analysis:** The calculated amounts of osteoblasts, osteoclasts, and FGF-2 expression data were used in the one-way ANOVA test. **Results:** FGF2 expression and the number of osteoblasts increased significantly after 7-day and 30-day of examination. On the other hand, the number of osteoclasts decreased. **Conclusion:** The combination of MPE and DFDBBX effectively increases FGF-2 expression and the number of osteoblast cells while decreasing the number of osteoclast cells on the alveolar ridge of Cavia cobaya.

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INTRODUCTION

The loss of alveolar bone support compromises stability, support, and comfort when placing prostheses or dental implants. Thus, maintaining the alveolar bone is carried out after tooth extraction to minimize residual ridge resorption (Radoczy-Drajko, 2021).

Alveolar bone loss can be caused by a variety of factors, including pathological factors, root canal treatment (endodontic), periodontitis, facial trauma, and aggressive actions during tooth extraction (Lin, 2019). The severity of the post-extraction healing assessment influences dentists with respect to aesthetic design when creating implant restorations or inserting conventional prostheses. However, this problem can be overcome by a socket-preserving procedure using graft material with or without a membrane barrier after tooth

extraction. During the process of alveolar bone regeneration, natural or synthetic graft materials are used to repair defects or deficiencies in tissue by providing an extracellular matrix in the form of a scaffold (Kang, 2019; Kim, 2020). Biomaterials used for bone regeneration must be biocompatible, biodegradable, and effective. Integrating biomaterials can improve strength and osteoconductive properties while also controlling resorption time (Ogueri, 2019). Demineralized freeze-dried bovine bone (DFDBBX) xenograft is one of the most commonly used graft materials. DFDBBX is a mineralized bone graft material that has been mineralized by immersing it in an acidic solution to remove minerals from the bone (Mahyudin, 2017). DFDBBX is commonly used as a graft in periodontal regenerative procedures in which the bone matrix is treated with hydrochloric acid to expose bone morphogenetic proteins (BMPs) capable of forming new bone (Kader, 2017).

Over the past several decades, various natural ingredients have been developed to support the bone formation process. Many tropical plants have interesting bioactivities with potential therapeutic applications. One of the most popular tropical plants is the mangosteen. Mangosteen (*Garcinia mangostana* L.) is a native fruit tree of Indonesia that grows easily in Southeast Asian countries such as Malaysia, Thailand, and Myanmar. According to phytochemical research, mangosteen peel extract (MPE) contains active ingredients such as xanthenes, flavonoids, saponins, and tannins. Xanthenes have been shown to have pharmacological effects such as antibacterial, antifungal, and anti-inflammatory properties (Aizat, 2019). MPE can also inhibit *Staphylococcus aureus* strains with a minimum inhibitory concentration (MIC) of 3.125 g/ml. Another study also showed that an 80% ethanol extract from MPE could inhibit the growth of *Porphyromonas gingivalis* W50, a major periodontal bacterium with a MIC of 3.91 mg/ml (Manjunatha, 2022). Bone regeneration is a complex biological process governed by cell-growth factor interactions. Blood vessels, osteoblasts, cementoblasts, and fibroblasts are among the cells involved in this regenerative process. Growth factors play an important role in all cellular functions, suggesting that exogenous agents could be used to repair bone tissue as an alternative therapeutic approach to bone repair.¹⁰ Growth factors are active polypeptides that influence epithelial, bone, and connective tissue cell proliferation, chemotaxis, and differentiation. Fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), transforming growth factor (TGF), osteoprotegerin (OPG), bone morphogenetic protein (BMP), and proteins found in cells and tissues are examples of polypeptides. Nuclear factor kappa B ligand receptor activator (RANKL) in both soft and hard tissues. Including nuclear factor kappa B ligand receptor activator (RANKL) in soft and hard tissues involved in bone regeneration (Kresnoadi, 2017). Among these polypeptides, FGFs are thought to be potential regulators of cell proliferation and wound healing, as well as having mitogenic properties. The best FGF group is FGF-2, which is important in the regulation of bone healing (Kigami, 2014). The purpose of this study, as stated above, is to investigate the clinical and microbiological effects of combining mangosteen peel extract (MPE) and demineralized freeze-dried bovine bone xenograft (DFDBBX) on the expression of fibroblast growth factor-2 (FGF-2), the amount of osteoblast, and the amount of osteoclast in the alveolar bone following Cavia cobaya tooth extraction. As a result, the combination of MPE and DFDBBX in the tooth extraction socket is expected to provide adequate bone graft strength, promote alveolar bone formation, and allow for proper denture and dental implant installation in the future.

5 MATERIALS AND METHODS

A randomized post-test control group design was used for the research. Sterile distilled water, MPE, DFDBBX, absolute alcohol, 70% alcohol, monoclonal antibody FGF-2 (Santa Cruz), immune staining kit reagent (Novocastra Leica), and a reagent for hematoxylin and eosin (HE) coloring were used in this research. The experimental animals were 56 male Cavia cobaya weighing 300–350 grams and aged 3–3.5 months. These samples were divided into two groups for

7-day (group A) and 30-day (group B) examinations, respectively. Each group consisted of twenty-eight animals that received four treatments. In each treatment, seven Cavia cobaya were used. The bottom right incisor was extracted. In group I, tooth sockets were filled with 25 grams of polyethylene glycol (PEG). Tooth sockets in group II were filled with 0.5 grams of DFDBBX and 24.5 grams of PEG. Tooth sockets in group III were filled with 0.5 grams of MPE and 24.5 grams of PEG. In group, tooth sockets were filled with a combination of 0.5 grams of DFDBBX, 0.5 grams of MPE, and 24 grams of PEG. Cavia cobaya's mandible was decalcified with ethylene diamine tetraacetic acid (EDTA) at 7 and 30 days. During this time, histopathology (HPA) and immunohistochemistry (IHC) tests were also performed. The results of the calculations are recorded and then tabulated. In addition, a statistical analysis was performed using the ANOVA test (analysis of multiple variances) from the calculated amount of osteoblast, osteoclasts, and FGF-2 expression data. Wh¹⁰ to compare the amount of data between different groups, multiple comparisons were performed using the Tukey HSD test.

RESULTS

A combination of MPE and DFDBBX was applied to the alveolar bone of Cavia cobaya extracted tooth sockets, with 56 Cavia cobaya divided into two groups, 7-day (group A) and 30-day (group B) examinations, respectively. Table 1 shows the results obtained. There was an increase in the expression of FGF-2 and the number of osteoblast cells after filling the Cavia cobaya tooth extraction socket with DFDBBX, MPE, or a combination of MPE and DFDBBX on the 7-day and 30-day Examination. While the number of osteoclasts decreased after control was achieved by filling the Cavia cobaya tooth extraction socket with DFDBBX, MPE, or a combination of MPE and DFDBBX on the 7-day and 30-day examination. Figure 1. shows the graph of mean and standard deviation of FGF-2 expression, the number of osteoblast cells, and the number of osteoclast cells in each treatment on the 7-day examination. On the seventh day of examination, it appeared that the treatment group with the combination of MPE and DFDBBX in the Cavia cobaya tooth extraction socket had the highest average of FGF-2 expression and the number of osteoblast cells and the lowest average number of osteoclast cells (Group IV). Figure 2. demonstrates the mean and standard deviation of FGF-2 expression, the number of osteoblast cells, and the number of osteoclast cells in each treatment group on the 30-day examination. The treatment group with the combination of MPE and DFDBBX in the Cavia cobaya tooth extraction socket had the highest average of FGF-2 expression and the number of osteoblast cells and the lowest average number of osteoclast cells (Group IV) at thirty days of examination. Figures 3 and 4 show microscopic features of FGF-2, osteoblasts, and osteoclasts expression in each treatment group on the 7th and 30th days of examination. The normality test yields $P = 0.000 < 0.05$, indicating that there are significant differences between the groups. A homogeneity test performed prior to the ANOVA test yielded $P = 0.167 > 0.05$ on the Levene test, indicating that we can proceed with the ANOVA test.

DISCUSSION

As the research progresses, a gel scaffold containing MPE and DFDBBX is applied to Cavia cobaya bone defects. A scaffold is a matrix or artificial structure that is used to promote cell invasion and physical support, resulting in cell proliferation and differentiation into functional tissues or organs of an organism (Vaidyanathan, 2023; Isola, 2022). Scaffold functions are coordinated to modify cell attachment and migration by transporting and defending cells in biochemical factors, activating nutrient diffusion into vital cells, and exerting certain mechanical and biological effects to modify cell phase behavior.¹⁴ Furthermore, scaffolds can provide structural integrity in the body before rupturing and leaving neo tissue (newly formed tissue subjected to mechanical stress).¹⁵ In vivo testing with DFDBBX, MPE, or both MPE and DFDBBX has been shown to increase FGF-2 and osteoblast activity.

Table 1.

Group	Number of samples	FGF-2 Expression (%)		Osteoblast cell number		Osteoclast cell number		
		Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	
A	I	7	3	0,79	4,5	1,13	19,29	2,88
	II	7	5,86	1,16	6,3	1,90	14,86	4,02
	III	7	7,29	1,46	8,43	2,44	14,71	1,50
	IV	7	10,71	1,77	9	2,45	13,71	2,43
B	I	7	7,29	2,22	12,43	1,62	10,43	2,23
	II	7	10,71	2,29	13,27	2,22	8,57	1,72
	III	7	13,71	2,43	15,71	0,98	6,57	1,62
	IV	7	16,29	1,80	16	1,83	5,14	1,46

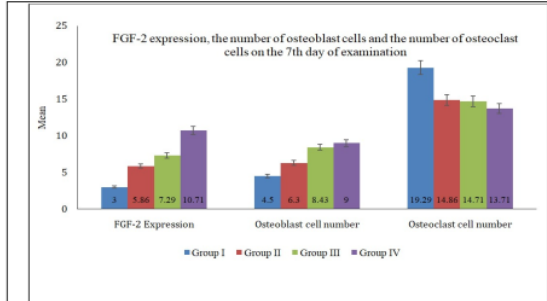


Figure 1. Graph of FGF-2 expression, number of osteoblast and osteoclast cells on the 7th day of examination

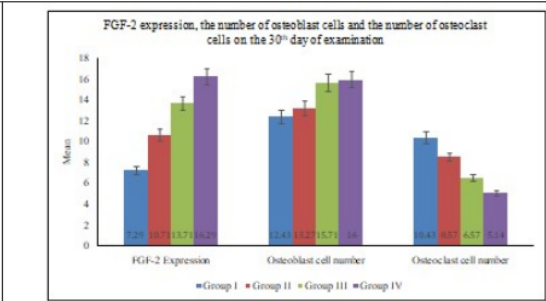


Figure 2. Graph of FGF-2 expression, number of osteoblast and osteoclast cells on the 30th day of examination

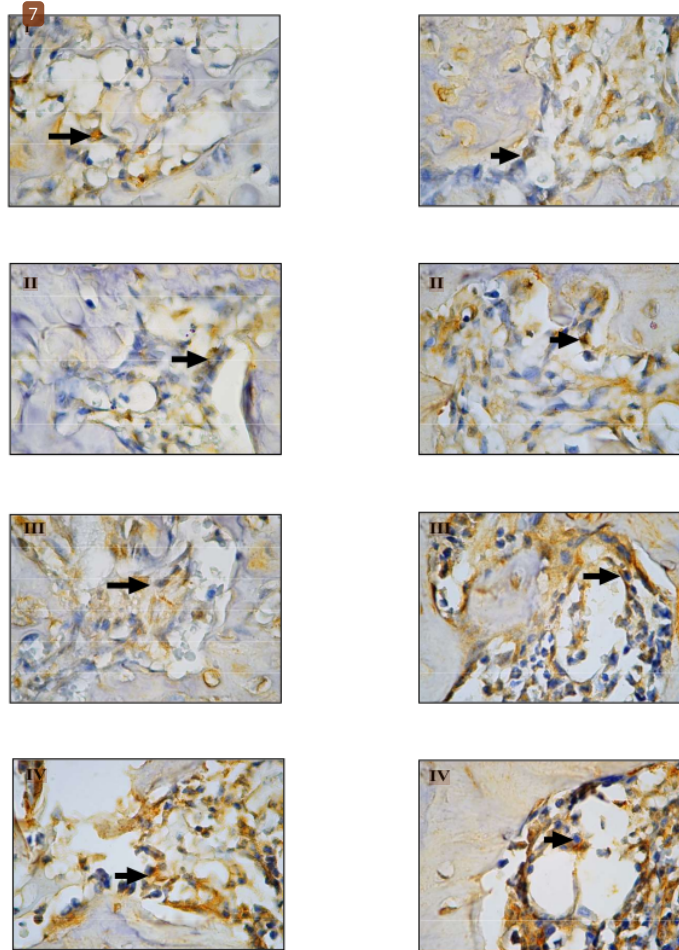


Figure 3. Microscopic picture on the 7th (left) and 30th (right) day of examination in each treatment group (I) PEG; (II) DFDBBX + PEG; (III) MPE + PEG; (IV) MPE + DFDBBX + PEG. Black arrow indicates of FGF-2 expression

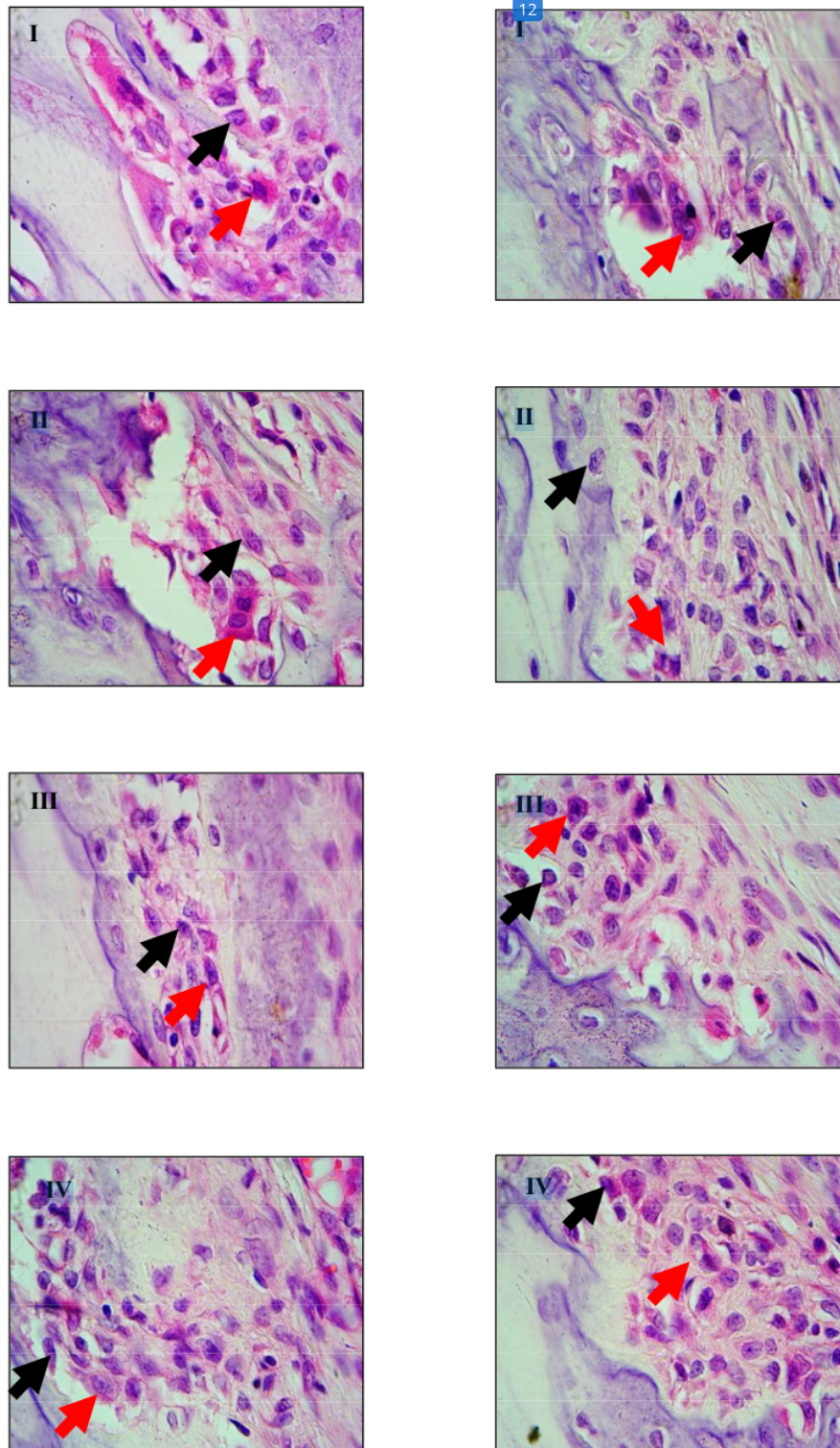


Figure 4. Microscopic picture on the 7th (left) and 30th (right) day of examination in each treatment group (I) PEG; (II) DFDBBX + PEG; (III) MPE + PEG; (IV) MPE + DFDBBX + PEG. Red arrow indicates of osteoblast cells and black arrow indicates of osteoclast cells

On the 7th and 30th days of examination, in vivo testing with DFDBBX, MPE, or both MPE and DFDBBX can increase FGF-2 and osteoblast cell numbers. Examinations were performed on day 7, when the osteoblast cells began to attach to the surface of the scaffolds (Motamedian, 2015). After scaffolds were added to animal tests, osteoblasts increased significantly during the first 20 days, and blood vessels formed by day 14, with examinations continuing until day 30 of the study (Kaniuk, 2020; Salim, 2015). The results of the study, which were observed on days 7 and 30, revealed that the DFDBBX, MPE, or combined MPE and DFDBBX treatment groups increased FGF-2 expression and the number of osteoblasts in comparison to the control group. The outcome was improved because it was transformed into an artificial structure for infiltration and bone replacement cells. An artificial structure that can maintain the mechanical stability of the tissue and restore the bone defect to its original shape is required as the tissue growth site for bone tissue regeneration (Kresnoadi, 2020). Conversely, the composition of the socket filling material can increase cell interaction and thus cell proliferation (Abate, 2022).

Various tests on the number of osteoblasts and FGF-2 expression at days 7 and 30 revealed an increase with all treatments. MPE and DFDBBX contain reactive amino and hydroxyl chains that can stimulate fibroblasts to proliferate, migrate, and produce interleukin, resulting in a decrease in osteoblasts in both the treated and control groups. Because the process began after the seventh day, the examination on day 30 revealed a significant increase over day 7. DFDBBX is a xenograft with excellent biocompatibility and bioactivity. Furthermore, DFDBBX has crystallographic and chemical properties that are similar to the structure of bones and teeth. Xenografts promote the growth of osteoblasts, fibroblasts, and endothelial cells (Jeong, 2019). Furthermore, because the inner surface, porosity, calcium ratio, hydroxyapatite, and other mineral factors of bovine bones are similar to those of human bone minerals, xenografts are known to have the ability to regenerate tissue through osteoconduction (Cunniffe, 2016). According to this study, the average FGF-2 expression and osteoblast cell numbers in post-extracted socket fillings using DFDBBX or MPE were lower than the combined MPE and DFDBBX group. The combination of MPE and DFDBBX is more stable because the composition of MPE can act as a bridge to enhance the binding efficiency between the amino acid molecules of MPE and DFDBBX in the tissue (Abate, 2022). According to the study's findings, after applying DFDBBX, MPE, or a combination of MPE and DFDBBX on the days 7 and 30, the number of osteoclast cells that were initially higher in the control group appears to have decreased. This is because DFDBBX, MPE, or a combination of MPE and DFDBBX play an important role in preventing inflammation, accelerating tissue wound healing, and decreasing the number of osteoclast cells.

The results show that the combination of MPE and DFDBBX promotes bone growth, particularly in extracted tooth sockets. The reason for this is that DFDBBX acts more as an osteoconductor in bone regeneration by providing an extracellular matrix in the form of a scaffold. MPE, on the other hand, according to the study's findings, the number of osteoclast cells that were initially higher in the control group appears to have decreased after applying DFDBBX, MPE, or a combination of MPE and DFDBBX on the 7-day and 30-day examination. This is due to the fact that DFDBBX, MPE, or a combination of MPE and DFDBBX play an important role in preventing inflammation, stimulating faster tissue wound healing, and decreasing the number of osteoclast cells. The findings show that the combination of MPE and DFDBBX had a synergistic effect on bone growth, particularly in extracted tooth sockets. The reason for this is that DFDBBX acts more as an osteoconductor in bone regeneration by providing an extracellular matrix in the form of a scaffold. MPE, on the other hand, has an osteoconductive effect because it stimulates FGF-2 and osteoblast proliferation.

CONCLUSION

Based on the findings, it can be concluded that the combination of MPE and DFDBBX effectively increases FGF-2 expression and the number of osteoblast cells while decreasing the number of osteoclast cells on the alveolar ridge of *Cavia cobaya*. More research is needed on biochemical markers of osteogenesis such as alkaline phosphate, osteocalcin, osteopontin, and fibronectin. This research is expected to be widely developed in the dental field, leading to the development of biotechnology and the production of patented products.

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